

FIG. 1A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS					
		1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05
Thr <sup>1</sup>	X-X-X-X-Thr <sup>1</sup> -X-X-X-X-X-Cys	1.92	1.32	0.54	0.34	0.07	0.04
Ser-Thr	X-X-X-X-X-Ser-Thr-X-X-X-X-X-Cys	0.11	0.05	0.01	0.00	0.01	0.00
Threonine <sup>1</sup> mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.26	0.10	0.07
Serine <sup>1</sup> mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01
Akt-Thr308-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr <sup>1</sup> -Phe-Cys-Gly-Thr-Pro (SEQ ID NO:1)	1.18	0.85	0.24	0.13	0.03	0.01
APPI1-Thr688-P	Asp-Ala-Ala-Val-Thr <sup>1</sup> -Pro-Lys-Lys-Arg-His-Leu-Ser-Lys-Cys (SEQ ID NO:2)	0.14	0.03	0.01	0.01	0.01	0.01
C3-P	Asp-Thr-Gln-Ile-Lys-Arg-Asn-Thr <sup>1</sup> -Phe-Val-Gly-Thr-Pro-Phe-Cys (SEQ ID NO:3)	1.71	1.13	0.39	0.22	0.03	0.02
CAK-Thr167-P	His-Gln-Val-Val-Thr <sup>1</sup> -Arg-Trp-Tyr-Arg-Cys (SEQ ID NO:4)	1.77	1.15	0.41	0.27	0.06	0.03
CAMIV-Thr186-P	His-Gln-Val-Leu-Met-Lys-Thr <sup>1</sup> -Val-Cys-Gly (SEQ ID NO:5)	1.79	1.36	0.63	0.40	0.09	0.05
CDC2-Thr167-P	Ile-Pro-Ile-Arg-Val-Tyr-Thr <sup>1</sup> -His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:6)	1.02	0.56	0.14	0.08	0.03	0.01
CDK2-Thr159-P	Gly-Val-Pro-Val-Arg-Thr <sup>1</sup> -Tyr <sup>1</sup> -His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:7)	1.88	1.79	0.51	0.44	0.08	0.04
p70S6K-Thr289-P	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr <sup>1</sup> -Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO:8)	1.99	1.44	0.82	0.39	0.08	0.04
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr <sup>1</sup> -Thr-Arg-Thr <sup>1</sup> -Phe-Cys (SEQ ID NO:9)	1.82	1.63	0.94	0.58	0.15	0.08
ERK2-P	Asp-His-Thr-Gly-Phe-Leu-Thr <sup>1</sup> -Glu-Tyr <sup>1</sup> -Val-Ala-Thr-Arg-Trp-Cys (SEQ ID NO:10)	1.56	1.18	0.51	0.30	0.07	0.04
Myc Ser58/62-P	Glu-Leu-Leu-Pro-Thr <sup>1</sup> -Pro-Pro-Leu-Ser <sup>1</sup> -Pro-Ser-Arg-Arg-Ser-Cys (SEQ ID NO:11)	0.11	0.05	0.03	0.02	0.02	0.02
P38-2P	Leu-Ala-Arg-His-Thr-Asp-Glu-Met-Thr <sup>1</sup> -Gly-Tyr <sup>1</sup> -Val-Ala-Thr-Arg-Cys (SEQ ID NO:12)	0.54	0.30	0.08	0.08	0.04	0.04
JNK2P	Ser-Phe-Met-Met-Thr <sup>1</sup> -Pro-Tyr <sup>1</sup> -Val-Val-Thr-Arg-Tyr-Tyr-Arg-Cys (SEQ ID NO:13)	1.49	0.44	0.12	0.07	0.03	0.02

100 100 100 100 100 100 100 100

**FIG. 1B**

PEPTIDE SEQUENCE	phospho-Thr Reactivity
XXXXXXS*XXXXXX	—
XXXXY*XXXX	—
XXXXXPXS*/T*PXR/KXXX (SEQ ID NO:14)	++
XXXXRSXS*XPXXXX (SEQ ID NO:15)	—
XXXXRSXSXPXXXX (SEQ ID NO:16)	—
XXXXXPXS*/T*PXXXXX (SEQ ID NO:17)	++
XXXXXPXS/TPXXXXX (SEQ ID NO:18)	—
XXXXXT*XXXXXX	+++
XXXXXXS/TXXXXXX	—
21 phospho-Thr peptides mixture	+++
38 phospho-Ser peptides mixture	—
30 phospho-Tyr peptides mixture	—
<b>NEB LIBRARY</b>	
X-X-X-X-D/E-X-X-S*-T*-X-X-X-X-X-C (SEQ ID NO:19)	+++
X-X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20)	++
X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21)	+++
X-X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22)	+++
X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:23)	+++
X-X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24)	+++
X-X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-X-C (SEQ ID NO:25)	+++
X-X-X-X-X-X-S*/T-P-X-X-X-X-X-X-C (SEQ ID NO:26)	++
X-X-X-X-X-T*-X-X-X-X-X-X-C	+++
X-X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-C (SEQ ID NO:27)	++
X-X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28)	—
X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-X-C (SEQ ID NO:29)	++
<b>ANTIBODY REACTIVITY</b>	
+++ very strong	ELISA O.D. > 2
++ strong	1 - 2
+ weak	0.2 - 1
- very little	< 0.2

**FIG. 1C**

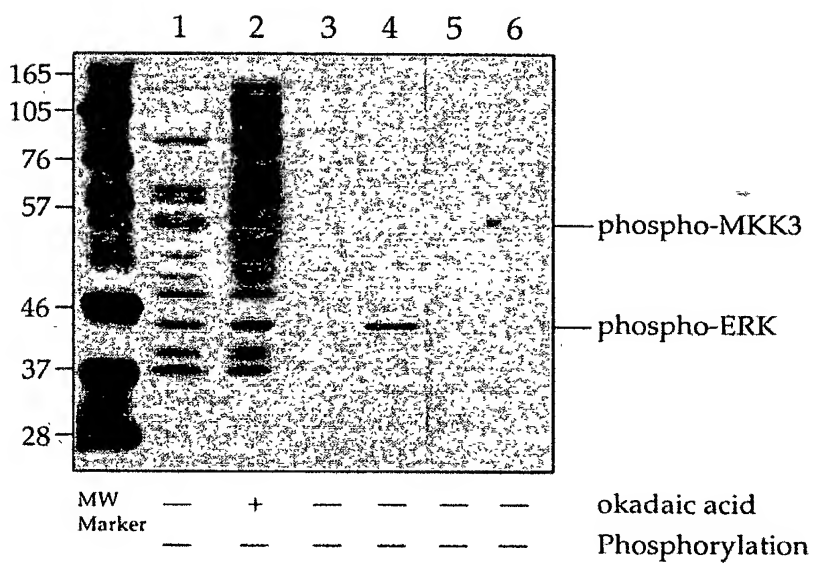


FIG. 1D

Fixed Amino Acid	-5-4-3-2-1 X X X X X Ser*/Thr* X X X X X Fixed AA position relative to phospho-Ser*/Thr*							+1+2+3+4+5		
	-4	-3	-2	-1	S*/T*	+1	+2	+3		
Ala	+	+	+	+		+	+	+		+
Cys	+	+	+	+		+	+	+		+
Asp	+	+	+	+		+	+	+		+
Glu	+	+	+	+		+	+	+		+
Phe	+	+	+	+		+	+	+		+
Gly	+	+	+	+		+	+	+		+
His	+	+	+	+		+	+	+		+
Ile	+	+	+	+		+	+	+		+
Lys	+	+	+	+		+	+	+		+
Leu	+	+	+	+		+	+	+		+
Met	+	+	+	+		+	+	+		+
Asn	+	+	+	+		+	+	+		+
Pro	+	+	+	+		+	+	+		+
Gln	+	+	+	+		+	+	+		+
Arg	+	+	+	+		+	+	+		+
Ser	+	+	+	+		+	+	+		+
Thr	+	+	+	+		+	+	+		+
Val	+	+	+	+		+	+	+		+
Trp	+	+	+	+		+	+	+		+
Tyr	+	+	+	+		+	+	+		+

10017465 .031002

**FIG. 2A**

PEPTIDE	SEQUENCE	1.00E+03	ANTIBODY DILUTIONS				1.00E+05	5.00E+05	1.00E+06
			5.00E+03	1.00E+04	5.00E+04	1.00E+05			
PXSP-P	X-X-X-X-X-Pro-X-Ser*-Thr*-Pro-X-X-X-X-X-Cys (SEQ ID NO: 27)	1.82	1.97	1.74	1.40	0.70	0.35	0.08	
Threonine mix	18 phospho-Thr peptide mix	1.97	1.37	0.67	0.36	0.13	0.07	0.05	
Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-X-Cys (SEQ ID NO: 28)	0.14	0.03	0.01	0.00	0.00	0.00	0.00	0.00
R18 Thr373-P	Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 30)	2.07	2.17	1.70	1.20	0.48	0.18	0.03	
MKK3-Thr-P	Ser-Val-Ala-Lys-Thr*-Met-Asp-Ala-Gly-Cys (SEQ ID NO: 31)	0.06	0.04	0.01	0.00	0.00	0.00	0.00	
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr*-Phe-Cys (SEQ ID NO: 9)	0.05	0.02	0.01	0.00	0.01	0.00	0.00	
p70 S6K-Thr389	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr*-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO: 8)	0.11	0.05	0.01	0.00	0.01	0.00	0.00	
cdk4-Thr172-P	Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Leu-Thr*-Pro-Val-Val-Val-Lys-Cys (SEQ ID NO: 32)	2.07	2.21	2.01	1.55	0.69	0.31	0.07	

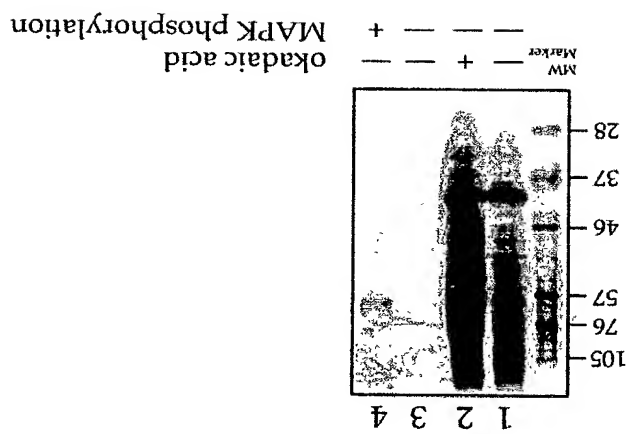
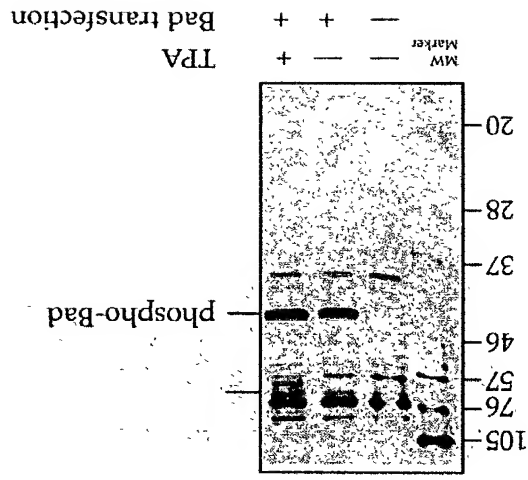


FIG. 2B

100-44603-03192

100-44603-03192



**FIG. 3B**

11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

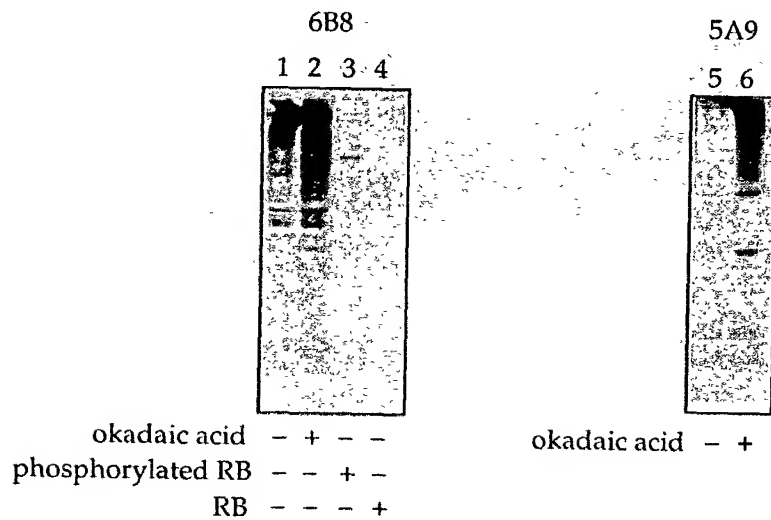


FIG. 4A

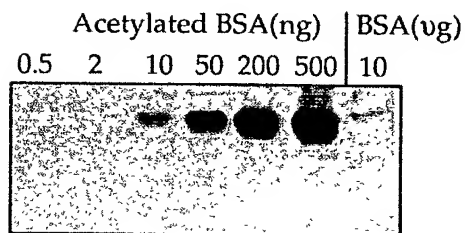
PEPTIDE	SEQUENCE	MONOCLONAL ANTIBODIES	
		6B8	5A9
Ser/ThrPro-P	X-X-X-X-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:26)	1.774	0.731
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr*-Pro-X-X-X-X-X-Cys (SEQ ID NO:27)	0.924	0.766
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:41)	0.02	0.063
ProXSer/ThrProXArg-P	X-X-X-X-X-Pro-X-Ser/Thr*-Pro-X-Arg/Lys-X-X-X-Cys (SEQ ID NO:42)	1.955	1.275
Thr-P	X-X-X-X-X-X-Thr*-X-X-X-X-X-X-Cys	0	--
Ser-P	X-X-X-X-X-X-Ser*-X-X-X-X-X-X-Cys	0.031	0.088
Ser/Thr	X-X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys	0.021	0.066
Tyr-P	X-X-X-X-X-X-Tyr*-X-X-X-X-X-X-Cys	0.023	0.072
Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser*-Pro-Leu-Arg-Ile-Pro-Gly-Cys (SEQ ID NO:43)	0.032	0.124
Rb (Thr373)-P	Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:30)	3.336	3.503
Rb (Thr373)	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:44)	0.02	0.073

11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

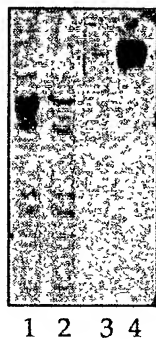
**FIG. 4B**



**FIG. 5A**



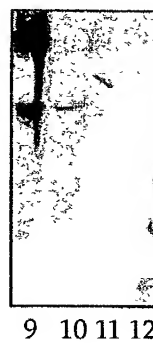
**FIG. 5B**



**FIG. 5C**

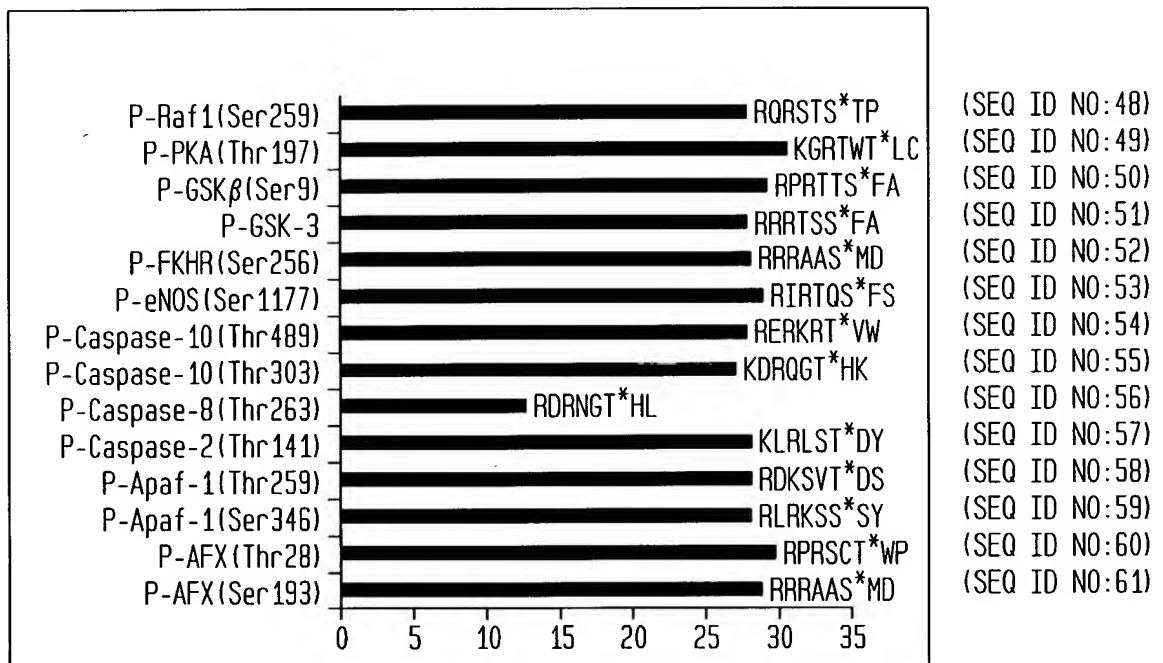


**FIG. 5D**



# FIG. 6

Signal to noise ratio of ELISA  
 readings using phospho-Akt substrate  
 antibody.



**FIG. 7**

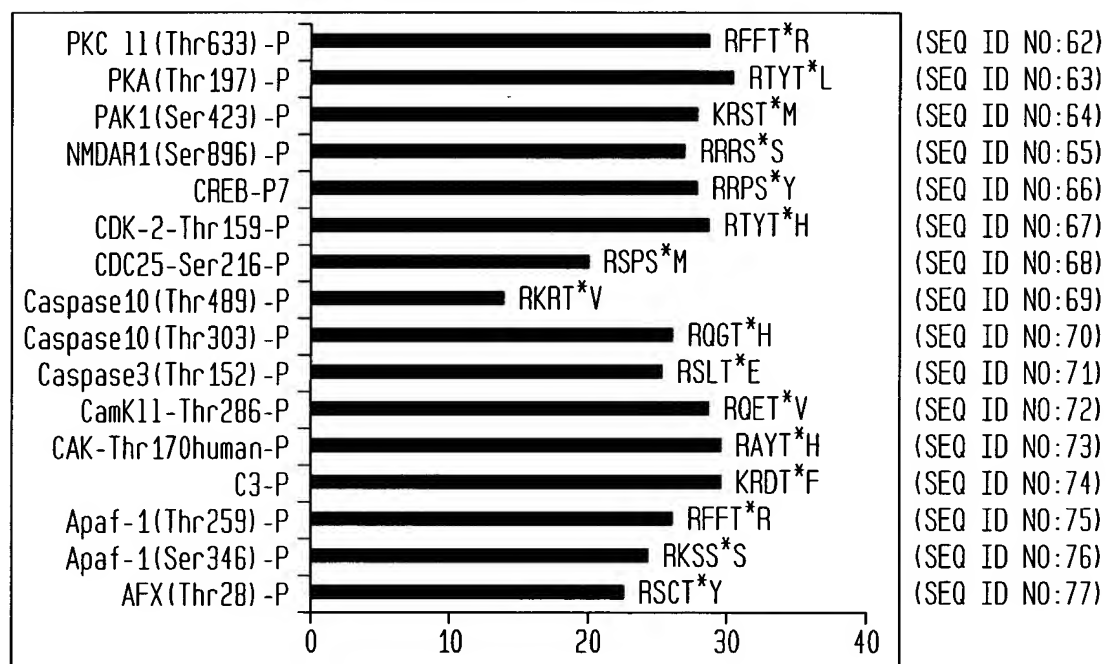
Western analysis of  
calyculin A-treated A431 cells  
using Phospho-Akt Substrate Anti-  
body.

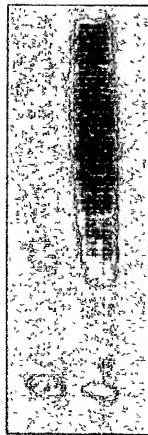


- +  
calyculin A

# FIG. 8

Signal to noise ratio of ELISA  
reading using phospho-PKA substrates  
antibody.

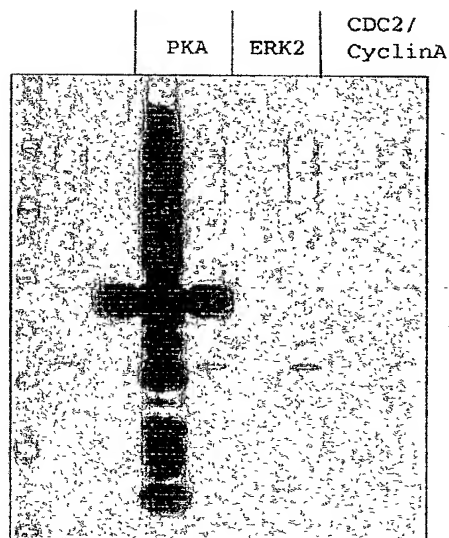




**FIG. 9**

Western analysis of  
calyculin A-treated A431 cells  
using Phospho-PKA Substrate  
Antibody.

- + calyculin A



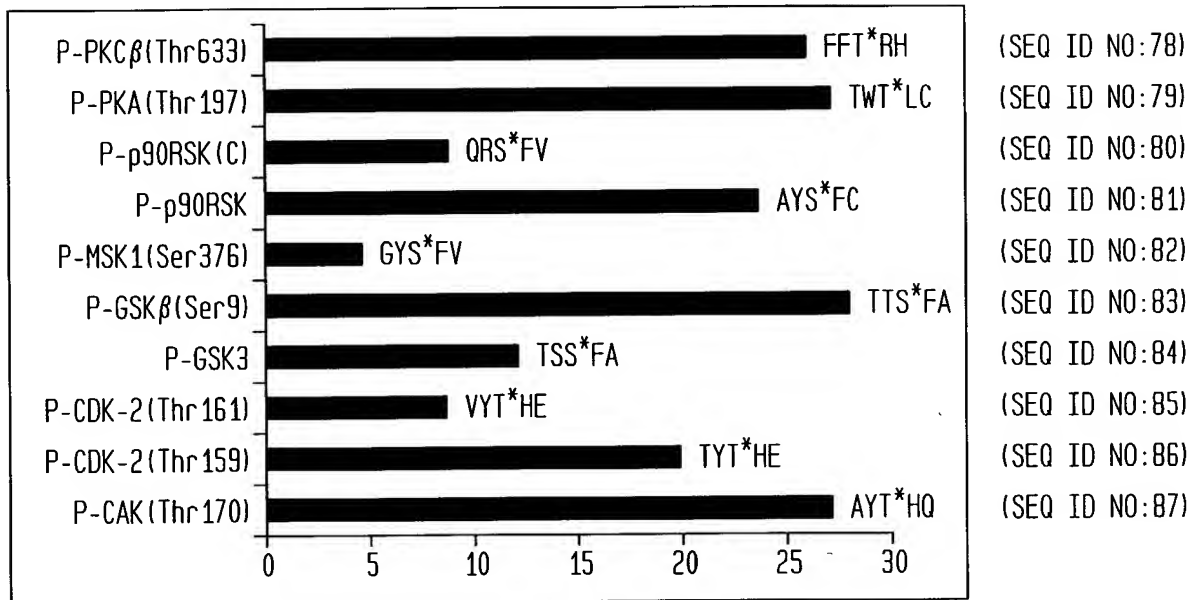
**FIG. 10**

### Western analysis of A431 cell extracts

+	-	+	+	-	+	-	+	Cell Extracts
-	-	-	+	-	-	-	-	PKI

# FIG. 11

Signal to noise ratio of ELISA  
reading using phospho-Serine/threonine  
phenylalanine antibody.





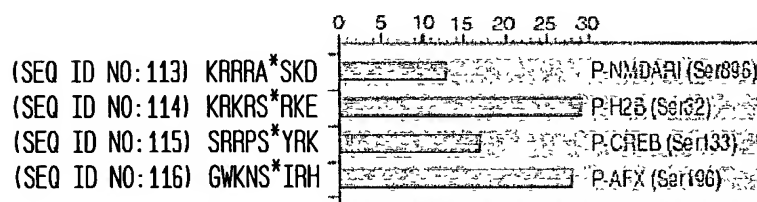
**FIG. 12**

Western analysis of calyculin  
A-treated A431 cells using phospho-  
Serine/phenylalanine substrates antibody.

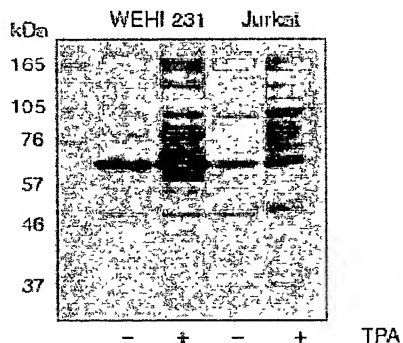


- +  
calyculin A

Signal to noise ratio of ELISA reading using a context-independent phospho-PKC consensus substrate motif antibody.

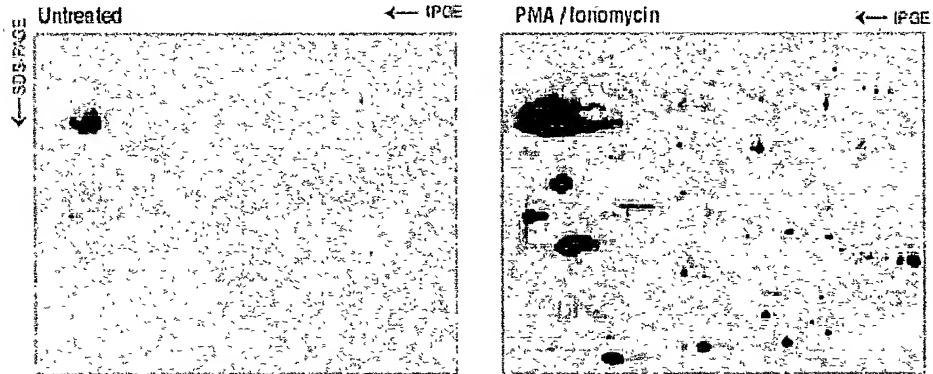


Western blot analysis using a context-independent phospho-PKC consensus substrate motif antibody.



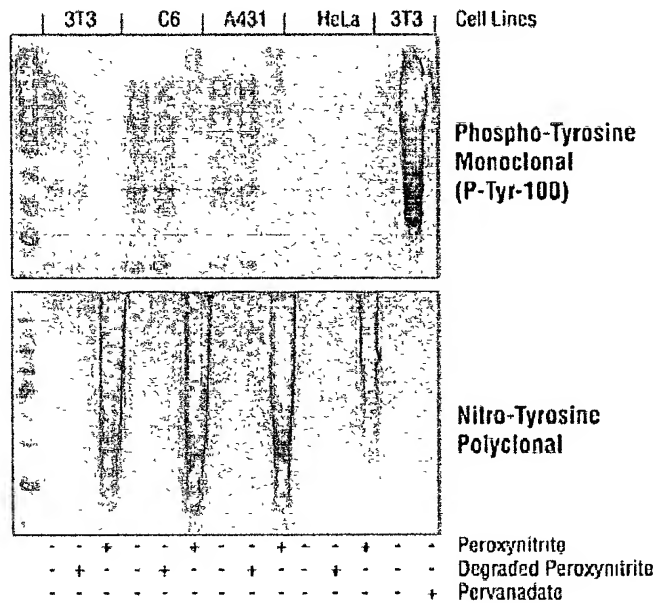
**FIG. 15**

Western blot analysis of whole cell lysates using a context-independent antibody specific for the phospho-PKC consensus substrate motif.



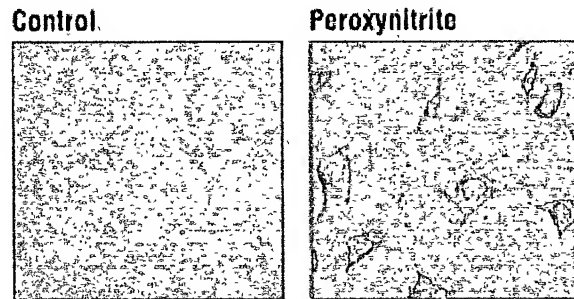
**FIG. 16**

Western analysis of whole cell lysates using a phosphotyrosine and nitrotyrosine - specific context-independent antibodies.



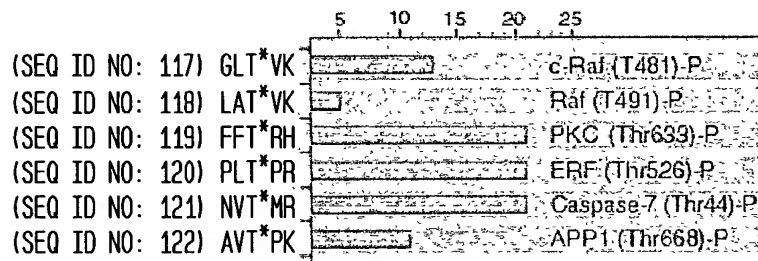
**FIG. 17**

Immunocytochemical staining of NIH/3T3 cells using a polyclonal context-independent antibody specific for nitrotyrosine (brown).



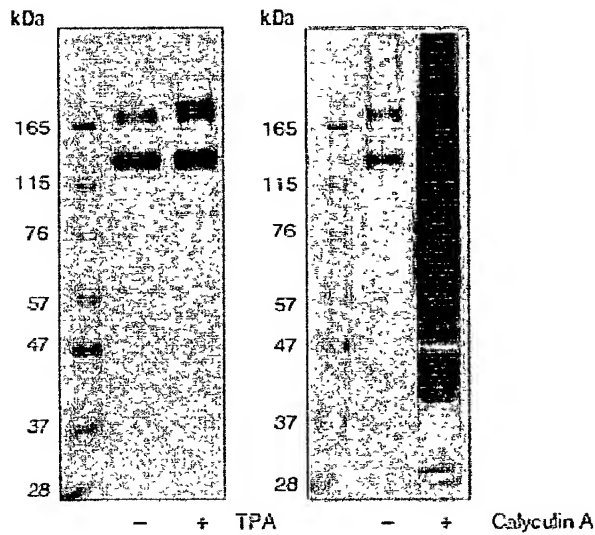
**FIG. 18**

Phosphothreonine-X-arginine motif-specific context-independent antibody ELISAs.



**FIG. 19**

Western blot analysis of Jurkat cell extracts using a context-independent antibody specific for the phosphothreonine-X-arginine motif.



**FIG. 20**

IHC staining of proteins containing phosphorylated threonine-X-arginine motifs in human breast carcinoma.

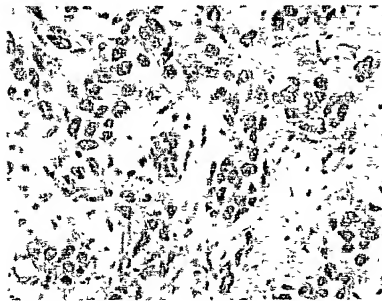


FIG. 21

Western blot analysis of calyculin A treated A431 cells, using a context-independent antibody specific for the phospho-14-3-3 binding motif #2 (phospho(Ser)-Arg-X-(Tyr/Phe)-X-pSer).

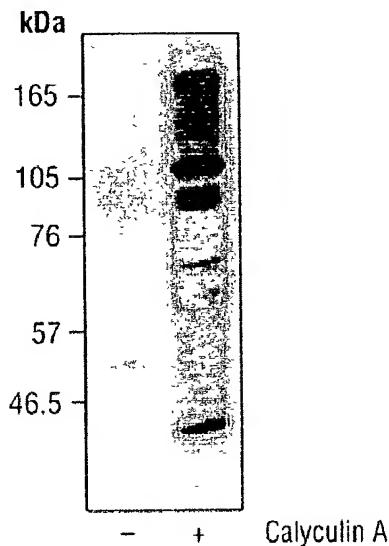
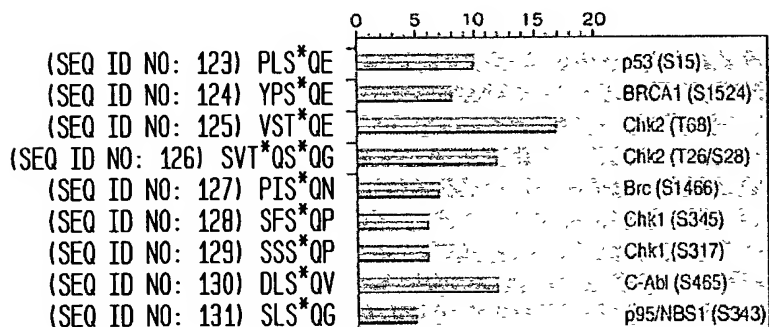


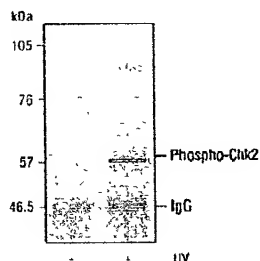
FIG. 22

Phospho-ATM/ATR consensus substrate motif-specific, context-independent antibody ELISAs.



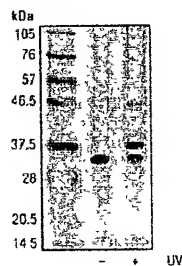
### FIG. 23

Western blotting of COS cell extracts using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.



### FIG. 24

Western blot analysis of UV treated COS cells, using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.



### FIG. 25

Phospho-14-3-3 binding motif-specific, context-independent monoclonal antibody ELISAs (T\* and S\* denote phospho-threonine and serine).

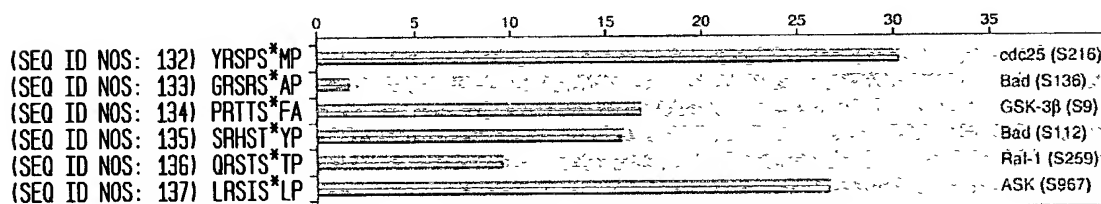


FIG. 26

Western blot analysis of calyculin A treated A431 cells, using a context-independent antibodies specific for phospho-14-3-3 binding motif #1.

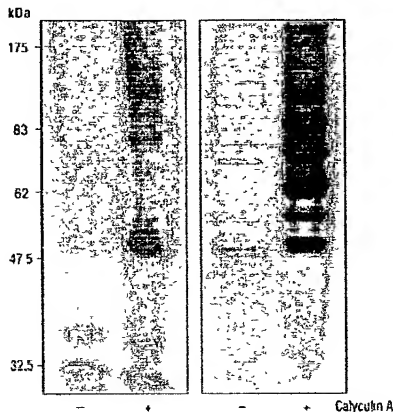
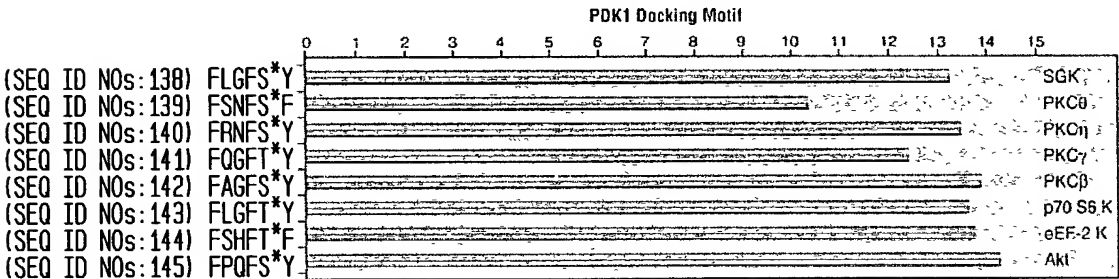


FIG. 27

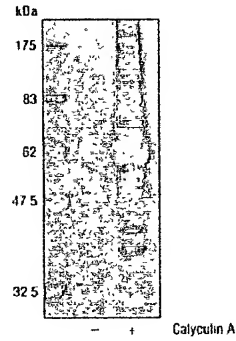
Phospho-PDK1 docking motif-specific, context-independent monoclonal antibody ELISAs. (T\* and S\* denote phospho-threonine and serine.)





**FIG. 28**

Western blot analysis of extracts from A431 cells using a monoclonal context-independent antibody specific for the phospho-PDK1 docking motif.



**FIG. 29**

Immunoprecipitation of extracts from NIH/3T3 cells using a monoclonal context-independent antibody specific for the phospho-PDK1 docking motif.

